High genetic variability in the mtDNA control region of a Northwestern Atlantic teleost, *Scomber scombrus* L.

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High genetic variability in the mtDNA control region of a Northwestern Atlantic teleost, *Scomber scombrus* L.

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ABSTRACT

Lambrey de Souza, J., J.-M. Sévigny, J.-P. Chanut, W. F. Barry, and F. Grégoire. 2006. High genetic variability in the mtDNA control region of a Northwestern Atlantic teleost, *Scomber scombrus* L. Can. Tech. Rep. Fish. Aquat. Sci. 2625: vi + 25 pp.

The left branch of the mtDNA control region was sequenced for 76 Atlantic mackerel (*Scomber scombrus* L.) sampled in the Gulf of St. Lawrence, the Gulf of Maine and off the North Carolina coast in an attempt to determine if the northern and southern populations of this species are genetically differentiated. Sequencing has revealed that the mtDNA control region is highly variable in this species. Indeed, 48 haplotypes were observed in the 76 individuals analyzed, and haplotype diversity values varied from 0.982 in the Gulf of Maine to 0.992 in the Gulf of St. Lawrence. The results of the AMOVA and of the phylogenetic analyses did not reveal differences between the northern and the southern populations. The results of the present study suggest that, if homing is a feature of the reproductive behaviour of the Atlantic mackerel, straying appears to be high enough to prevent genetic divergence between the two populations in the Northwest Atlantic. However, the very high variability of the studied mtDNA fragment and the small sample size used may have prevented the detection of genetic organization of the species.

RÉSUMÉ

Lambrey de Souza, J., J.-M. Sévigny, J.-P. Chanut, W. F. Barry et F. Grégoire. 2006. High genetic variability in the mtDNA control region of a Northwestern Atlantic teleost, *Scomber scombrus* L. Can. Tech. Rep. Fish. Aquat. Sci. 2625: vi + 25 p.

La branche gauche de la zone de réplication de l'ADN mitochondrial a été séquencée chez 76 maquereaux bleus (*Scomber scombrus* L.) échantillonnés dans le golfe du Saint-Laurent, le golfe du Maine et au large de la côte de la Caroline du Nord dans le but de déterminer si les populations nord et sud de cette espèce sont génétiquement différenciées. Le séquençage a révélé que la zone de réplication de l'ADN mitochondrial est très variable chez cette espèce. En effet, 48 haplotypes ont été détectés chez les 76 individus étudiés et les valeurs de diversité des haplotypes ont varié de 0.982 pour le golfe du Maine à 0.992 pour le golfe du Saint-Laurent. Les résultats des analyses de variance moléculaire (AMOVA) et des analyses phylogénétiques n'ont révélé aucune différenciation génétique entre les populations nord et sud. Les résultats de la présente étude suggèrent que si la phylopatrie est une caractéristique du comportement reproducteur du maquereau, le niveau des échanges entre les populations nord et sud est suffisamment élevé pour empêcher la divergence génétique des deux populations. Par ailleurs, la très grande variabilité du fragment d'ADN mitochondrial étudié et la faible taille des échantillons peuvent avoir empêché la détection d'une organisation génétique chez cette espèce.

INTRODUCTION

A characteristic of the marine environment is its propensity for long-distance dispersal of many species. Although ocean circulation patterns, sea-floor topology, and other geographic features provide opportunities for the isolation and differentiation of some species, most of the world's oceans lack obvious barriers to migration and dispersal. In addition, life histories of several marine species are characterized by at least one stage that is potentially widely dispersing. Several species have evolved extended pelagic larval stages and/or high migratory capabilities as juveniles or adults, resulting in widespread ocean dispersal. These characteristics reduce the potential for geographic differentiation between distant populations (e.g., Graves 1998; Thorrold et al. 2001).

A large number of migratory marine fish species that have broad geographical distributions can exhibit significant levels of population subdivision if they show post-dispersal spawning fidelity to natal areas (Ruzzante et al. 1998), a phenomenon known as natal homing. Such phylopatric behaviour (tendency of an organism to stay in, or return to, its home area) appears to be quite common among aquatic species. The salmon's natal homing ability, for example, is one of the best-studied migratory patterns among animals (Cury 1994). Many marine fish species such as Atlantic herring (*Clupea harengus*), capelin (*Mallotus villosus*), and Atlantic cod (*Gadus morhua*) exhibit homing migrations (Wheeler and Winters 1984, Cury 1994, O'Connell et al. 1998, Waters et al. 2000a, Robichaud and Rose 2001).

The Atlantic mackerel (*Scomber scombrus*) is an epipelagic and mesodemersal species of Scombridae that is most abundant in cold and temperate shelf areas. In the Northwest Atlantic, its distribution extends from Labrador to Cape Hatteras. The species has been divided into two populations since the 1950s (Sette 1950). Fish of the southern population move inshore from mid-March to mid-April towards the Virginia, Maryland and New Jersey coasts. Until mid-May, they migrate northward and spawn until June along the coast of New Jersey and in the southern Gulf of Maine. During the summer, they feed in the Gulf of Maine (MacKay 1967). In mid-May, fish from the northern population move inshore towards the southern New England and southern Nova Scotia coasts (Sette 1950, MacKay 1967, Maguire 1981). These individuals migrate northeastwards along the Scotian Shelf. The most important component of the northern population enters the southern Gulf of St. Lawrence, where it spawns from early June to mid August. Other minor spawning sites for the northern population may also include the Newfoundland coast and the Scotian Shelf (Maguire et al. 1987). The fish of the northern population feed in the Gulf of St. Lawrence and off the Newfoundland coast until October (MPO 2001). The northern and southern populations migrate out of their respective spawning grounds at the beginning of autumn, move southwards along the United States coast and intermingle between longitudes 65° and 73°W, mostly over Georges Bank. They gradually move offshore to over-winter in deeper waters. Both populations are exploited on their spawning grounds in summer and in a winter–spring fishery (Overholtz et al. 1991), when they are mixed.

Characteristics of the Atlantic mackerel life cycle suggest that the exchange of individuals between the northern and southern populations may be relatively restricted before they reach the adult stage. Indeed, larvae hatch only 90 to 120 hours after fertilization (Studholme et al. 1999) and drift passively with surface currents generated mostly by wind. They develop fins when they reach 8-10 mm in length (Sette 1943). Since Atlantic mackerel distribution during the larval period is largely determined by wind strength and direction, it can be assumed that dispersal may not be important during this relatively short phase of the life cycle. Similarly, the winter migration of juvenile Atlantic mackerel in their first year is not extensive enough to ensure the mixing of individuals from both populations. Juveniles migrate later than adults and move to deeper waters only in late autumn. Juveniles from the northern region stay to over-winter on the Scotian Shelf. Because of the geographic separation of the two main spawning sites and the early growth patterns, it can be hypothesized that genetic differentiation could take place between Atlantic mackerel of the northern and southern regions if a strong homing behaviour exists in this species.

Sette (1950) was the first to address the question of differentiation in Atlantic mackerel. He hypothesized the existence of a southern and a northern population in the Northwest Atlantic based on studies of size composition in samples of commercially caught individuals collected daily from 1926 to 1936. He observed differences in length frequencies and age–length relations between individuals from the two populations, the southern individuals being smaller at age than their northern counterparts. He also noticed that spring commercial landings in New England consisted of both northern and southern individuals. The year-to-year consistency of tagging results, the absence of specific year-classes in either of the two populations, and the observation of the two main spawning sites in the Gulf of Maine and the Gulf of St. Lawrence led the author to suggest the existence of two distinct groups (contingents) of Atlantic mackerel in the Northwest Atlantic. Although Sette (1950) did not consider the two populations to be genetically distinct, he mentioned this possibility, specifying that the data and techniques available at the time did not allow this hypothesis to be tested.

Since the work of Sette (1950), different approaches have been used to test the hypothesis of differentiation of the two populations of Atlantic mackerel in the Northwest Atlantic. The comparison of meristic characters (MacKay and Garside 1969) and the analysis of otolith growth patterns (Simard et al. 1992) and shape (Castonguay et al. 1991) did not reveal significant differences between the two populations. The null hypothesis of panmixia between the northern and southern populations could not be rejected with the analyses of protein polymorphism, although some genetic difference between year-classes was suggested (MacKay 1967, Maguire et al. 1987).

Studies based on the use of artificial and natural tags have confirmed winter mixing of the two populations, originally shown by Sette (1950) off the coast of the United States, but could not quantify exchange rates between the two regions (MacKay 1967, Beckett et al. 1974, Parsons and Moores 1974, Isakov 1976). Two of the numerous tagging studies were carried out long enough to provide insights into the phylopatric spawning behaviour of Atlantic mackerel (Stobo 1976,

Waters et al. 2000b). In both studies, some tagged Atlantic mackerel were recoved in the tagging areas a year later. The authors concluded that phylopatric behaviour occurred in this species.

Natal homing is extremely difficult to demonstrate in marine organisms because the natal origins of adults are almost invariably unknown. This lack of knowledge is primarily due to the difficulty of conducting mark–recapture studies in species that are characterized by the production of large numbers of offspring that suffer high initial mortality rates (Thorrold et al. 2001). However, mtDNA is advantageous for such analyses because this molecule accumulates mutations more rapidly than several single-copy nuclear genes (e.g., allozymes). Because mtDNA shows haploid inheritance, the effective population size is one-quarter that of nuclear loci. Therefore, this marker is sensitive to divergence through drift. By using a fragment of mtDNA, the control region, to distinguish between spawning populations of *S. scombrus* in the Northeast Atlantic, Nesbo et al. (2000) tentatively demonstrated the existence of homing behaviour in this species. Recently, the analysis of a 272 bp fragment of the mitochondrial control region has revealed that mackerel from the eastern Mediterranean Sea are clearly separated from those of the western Mediterranean Sea, which could not be differentiated from those of the eastern Atlantic Ocean (Zardoya et al. 2004).

The objective of the present study was to determine if Atlantic mackerel from northern and southern regions in the Northwest Atlantic belong to genetically differentiated groups. The hypothesis that the species exhibits a phylopatric behaviour that leads to genetic differentiation was tested by sequencing a fragment of the mtDNA control region.

MATERIAL AND METHODS

Sampling

Atlantic mackerel were collected by trawl and gillnet fishing from various sites in 1998 and 2001, mostly in locations where the northern and southern populations are separated during the summer. Two samples of the northern population were collected in the Gulf of St. Lawrence during the 1998 (GL98) and 2001 (GL01) summer spawning seasons. One sample was collected in the Gulf of Maine one month after the 1998 spawning season (GM98), and one sample was collected off the North Carolina coast in winter 2001 (NC01) (Table 1; Fig. 1). The fish sampled off the North Carolina coast were collected during two NOAA (National Oceanic and Atmospheric Administration) survey cruises. The other Atlantic mackerel were collected from commercial fisheries.

All the fish were frozen onboard and sent frozen to the Maurice Lamontagne Institute (MLI) for analysis. Tail muscle tissue was taken from the left side of the fish immediately after thawing and was preserved in 95% ethanol that was replaced after 24 hours.

Specimen selection

Age (otolith readings), length, sex, and maturity stage (Maguire 1981) were determined on several specimens from each sample. Only immature individuals were available for the NC01 sample whereas spawning, post-spawning, and immature Atlantic mackerel were included in the GL01 sample. Specimens from the Gulf of Maine (GM98) and the Gulf of St. Lawrence (GL98) were selected regardless of their age and comprised several year-classes and maturity stages. A total of 76 individuals were used for the description of the mtDNA characteristics and in the determination of haplotype phylogeny. However, the age of one individual of haplotype 4 in the GM98 sample could not determined. Therefore, the AMOVAs are based on 75 individuals (Table 1).

DNA isolation, amplification, and sequencing

Total DNA was extracted using Qiagen's DNeasy Tissue Kit for DNA purification from animal tissues. The amplification of the left branch of the mtDNA was carried out on a Perkin Elmer DNA thermal cycler, using Lee's A and E primers (Lee et al. 1995). Amplifications were carried out in 50 µl final volume containing 10 to 100 ng template DNA, 1 X PCR buffer, 1.25 mM $MgCL_2$, 50 µM dNTP, 0.5 µM of each primer, and 0.5 U Taq-polymerase. The reactions were submitted to an initial 5-minute denaturation at 95°C and then to 40 cycles with denaturation at 93°C for 30 seconds, primer annealing at 50°C for 1 minute, and extension at 72°C for 2 minutes, followed by a final 10-minute extension at 72°C. Each sample was amplified in 10 replicates (500 µl total) and then extracted on a 2% low melt agarose gel using Qiagen's QIAquick gel extraction kit.

Sequencing was carried out in two different laboratories. Individuals of the GM98 and GL98 samples (n = 40) were analyzed on an ABI Prism 377 sequencer at the University of Ottawa. The other samples were sequenced at MLI using an ABI prism 310 sequencer. In order to test the sequencers' reliability, two GM98 individuals were also sequenced at MLI using the protocol described below. There was no difference between the sequencing results of the two laboratories. The procedure used at MLI was as follows. Both strands of the amplified product were sequenced on an ABI prism 310 sequencer using Lee's A and E primers (Lee et al. 1995) and the BigDye™ Terminator Sequencing Ready Reaction version 3.0 kit (Applied Biosystems). Sequencing was carried out following the manufacturer's instructions. Sequencing reactions consisted of 200 ng DNA, 8 µl BigDye version 3.0, 3.2 pmol of primer. Water was added to obtain a final reaction volume of 20 µl. Sequencing reactions were submitted to the same temperature parameters as those mentioned above for the amplification. Unincorporated dye terminators were washed using the DyeEx Spin Kit.

Sequences of the mtDNA control region were also obtained for two individual Atlantic mackerel from the Northeast Atlantic (European). The first sequence was obtained from Nesbo et al.

(2000) (GenBank accession number AJ132395) and the second one from Penzo and Paternallo (unpublished data; University of Padua, Via 8 Febbrario, 2-35122 Padova,Italy) (GenBank accession number AF149835).

Data analysis

Sequences were aligned using the Clustal ver. 1.8 software (Thompson et al. 1997) and alignment gaps were checked visually. The Arlequin ver. 2.000 software package (Schneider et al. 2000) was used to evaluate polymorphism in the sequences. The transversion–transition (Tv/Tr) ratio was set to 2:1, and the deletions were weighted equally to the transitions. These settings were used in the calculation of a matrix of Euclidian distances between mtDNA sequences in order to identify similar haplotypes.

Population variability was estimated by haplotype diversity (H) and nucleotide diversity (π_n) (Nei 1987). In a randomly mating population, H and π_n are estimates of heterozygosity at the gene and nucleotide levels respectively (Nei and Kumar 2000). Several common and molecular diversity indices were calculated to estimate sequence variability, such as the number of haplotypes, their relative frequencies, and the relative nucleotide composition.

Two approaches and additional tests were used to investigate the genetic structure of the Atlantic mackerel populations. First, analyses of molecular variance (AMOVA) were carried out on all year-classes within northern and southern regions without taking the specific sampling location within each region into consideration. This analysis incorporates frequencies of nucleotide differences between haplotypes into an analysis of variance for molecular data to estimate variance components within and between year-classes (Excoffier et al. 1992). Although resembling a hierarchical two-level ANOVA, this method has the advantage of using different evolutionary models regarding base pair nucleotide mutations to estimate different genetic distances for the calculation of the variances. The genetic variance components and the hierarchical F-statistic analogues were calculated and tested for significance using the 10 000 permutation method (Excoffier et al. 1992). The resulting ϕ-statistics give estimates of variance between populations attributable to non-random mating. The Tamura and Nei evolutionary model (Tamura and Nei 1993) and a gamma correction set to $\alpha = 0.40$ (Nesbo et al. 2000) were used. Other evolutionary models (e.g., Tajima, Kimura 2P distance, pairwise) and gamma corrections set to $\alpha = 0.9$ and $\alpha = 0.6$ were tested but showed no visible differences from the selected settings; therefore, these results are not presented and discussed further.

The second approach involved assessing the population structure of the Northwest Atlantic mackerel population through phylogenetic analyses. The concept behind the phylogenetic approach is that if the northern and southern populations of Atlantic mackerel are genetically differentiated, haplotypes in each population should be more related to each other than to those of the other population. Therefore, groupings of individuals on the resulting phylogenetic trees should correspond to the northern and southern populations.

Different trees were tested to describe the phylogenetic relations between individuals. These relationships were first estimated using the neighbour-joining algorithm (gamma corrected with α=0.40) implemented in PAUP 4b8 (Swofford 2000). It was impossible to reconstruct a comprehensive phylogeny of the selected individuals given the high haplotype diversity. Computer buffer capacity was reached with 32 700 trees, and consensus trees presented a great amount of homoplasies (convergent site changes or reverse mutations). The presence of few informative sites and homoplasies in mtDNA sequences often generate a good number of equally parsimonious trees (O'Corry-Crowe et al. 1997). To circumvent this difficulty, a maximum likelihood tree was constructed between individuals of samples NC01 and GL01 using the software PAUP 4b8 (Swofford 2000). The procedure adopted was as described by Hall (2001) with the optimality criterion set to "distance," gamma corrected with $\alpha = 0.40$, application of the Tamura and Nei evolutionary model (Tamura and Nei 1993) and 1 000 bootstrap replicates. This tree was rooted with inclusion of the sequences of two individuals from the Northeast Atlantic.

The relationships between haplotypes were described using a minimum spanning network constructed with Minspnet (Excoffier and Smouse 1994) as implemented in Arlequin ver. 2.000 (Schneider et al. 2000). The Tamura and Nei evolutionary model (Tamura and Nei 1993) and a gamma correction set to $\alpha = 0.40$ was used.

Two additional analyses were conducted in support of the preceding ones. The extent of heterogeneity in haplotype frequency distributions between males and females and among year-classes within samples and within regions was tested for significance using the χ^2 Monte Carlo randomisation procedure (Roff and Bentzen 1989) as implemented in the MONTE program of the REAP software (McElroy et al. 1992). Pairwise comparisons of ϕ_{ST} values were also calculated for all samples.

RESULTS

General results

The sequenced fragments were 316 bp long and correspond to the left branch of the mtDNA control region. The alignment of the 76 sequences revealed the presence of four conserved regions located at positions 1 to 17, 115 to 141, 217 to 230 and 262 to 292. These conserved regions separate four regions of higher variability, with the most variable being at position 142 to 216 (Fig. 2).

In the present study, 78 polymorphic sites were detected. They were composed of 49 transitions, 8 transversions, and 27 indels. The relative nucleotide composition of the sequenced fragment was as follows: A, 38.77%; T, 27.07%; C, 19.91% and G, 14.25%. The strong bias against

guanine is characteristic of mitochondrial genes as is the higher quantity of A and T over C (Meyer 1993).

The 78 variable sites observed in mtDNA defined a total of 48 haplotypes in the seven samples analyzed (Table 2). The northern and southern populations of Atlantic mackerel were characterized by a large number of rare haplotypes and a few more common ones. Haplotype 4 was the only one observed in samples from the Gulf of St. Lawrence ($N = 2$), the Gulf of Maine ($N = 1$) and off the coast of North Carolina ($N = 1$). However, this haplotype was not observed in all sampled years (Table 2). Each sample presented a large number of singletons: haplotypes 16 to 25 for NC01, haplotypes 26 to 34 for GM98, haplotypes 35 to 41 for GL98, and haplotypes 43 to 49 for GL01. Haplotypes 6, 9, 10, and 14 were only present in the northern population while haplotypes 7 and 12 were observed only in the southern population. A total of nine haplotypes were shared between the two populations of Atlantic mackerel (Table 2). The single haplotype from the Northeast Atlantic mackerel was not observed in the samples from the Northwest Atlantic.

Haplotype diversity, which indicates the probability that any two randomly chosen haplotypes are distinct from one another, was estimated to be 0.984 in the Northwest Atlantic and varied between 0.982 in the GM98 sample and 0.992 in the GL01 sample. These very high values indicate the presence of a large number of haplotypes within each sample. The nucleotide diversity (π_n) measures the pairwise within-population diversity as a function of haplotype diversity and the pairwise divergence between haplotypes. The nucleotide diversity value varied from 0.026 in the GL01 sample to 0.029 in the NC01 sample, with an overall value of 0.03 over the Northwest Atlantic (Table 3).

Population structure

Sample GM98 was collected in July after the spawning season. At this time of the year, samples collected in the Gulf of Maine may include individuals from both the northern and the southern populations. The presence of individuals of unknown origin in this sample may mask any population structure if such a structure exists. Therefore, tests assessing the population structure were carried out with and without the GM98 sample.

Analysis of molecular variance (AMOVA)

The results of the first AMOVA carried out on all year-classes within northern and southern regions showed that no genetic partition could be attributed to differences between regions. A low proportion (4.23%) of the total variance was due to differences between year-classes within northern and southern regions (Table 4). Only the variance associated with this component (between year-classes within regions) was significant ($p > 0.037$), whereas variance values associated to the two other components (between northern and southern regions; between individuals within year-classes) were not significant. However, although not significant, it is noteworthy that most of the variance is explained by genetic differences between individuals

within year-classes (97.37%). Discrimination of haplotypes between year-classes was low (ϕ_{SC} = 0.042).

The results of the second AMOVA carried out without the GM98 sample also showed that no genetic partition could be attributed to differences between regions. Once again, most of the haplotype diversity (96.22%) was found between individuals within year-classes (Table 4). Very small proportions of the variance were due to differences between year-classes within regions (2.94%) and between regions (0.84%). In this test, none of the three variance components were significant.

Haplotype phylogeny

The maximum likelihood analysis on the 1999 year-class of samples NC01 and GL01 failed to generate a tree showing geographical partitioning between northern and southern samples. Indeed, haplotypes from both samples can be found on a same branch of the tree (Fig. 3). Haplotype relationships were inferred from a minimum spanning network. The overall frequencies and relationships between haplotypes are presented in Fig. 4. The calculation parameters yielded null distances between a few haplotypes, thus pooling them on the network. The main feature of this network is the total absence of any structure associated with the sampling sites: the network cannot be nested into geographical locations corresponding to the northern and southern populations of Atlantic mackerel. Another feature of this network is the presence of two closely related star-like phylogenies with a central haplotype from which several rarer haplotypes extend. A few distant abundant haplotypes can be linked via rare haplotypes to these two central phylogenies. The most abundant haplotypes (haplotype 4 and the combination of haplotypes 2 and 47) are the most distant from the star-like phylogenies.

On the network, the haplotype from Northeast Atlantic mackerel is closest to haplotype 27. Consequently, the most ancestral state representing the common ancestor to western and eastern Atlantic mackerel must be part of the left star-like phylogeny. From this ancestral state, haplotypes have evolved towards recent and more frequent haplotypes towards the right of the network. The network did not show any further structuring when only considering fish sampled on their respective region during spawning season.

Homogeneity test

Possible difference in haplotype frequency distribution between sexes was tested for all males and all females pooled without considering the sampling sites. There was no difference in haplotype frequency distribution between males and females (χ^2 = 51.99; p = 0.146). Therefore, all additional tests were carried out without taking sex into account.

Tests of haplotype frequency distribution between the year-classes of the Gulf of St. Lawrence samples revealed no significant difference between the 1995, 1996 and 1999 year-classes (χ^2 =

53.27; $p = 0.176$). For this test, the three individuals of the 1994 year-class were not included (Table 5). A homogeneity test revealed a significant difference only when the 1995 and 1996 year-classes were considered ($\chi^2 = 18.00$; $p = 0.001$).

Similarly, no significant difference was detected between the three year-classes sampled in the southern region, i.e., the Gulf of Maine and North Carolina ($\chi^2 = 62.54$; $p = 0.256$). Significant differences were observed between the 1996 and 1997 year-classes sampled in the Gulf of Maine in 1998 ($\chi^2 = 18.00$; $p < 0.001$).

The detection of heterogeneity between year-classes limits the number of tests that can be carried out to detect geographic patterns of differentiation. However, homogeneity tests can be carried out on individuals of the same year-class collected in the different regions. Comparison of the 1999 year-class collected in the Gulf of St. Lawrence and off the North Carolina coast did not reveal a significant difference between the two regions ($\chi^2 = 29.93$; $p = 0.370$). Marginally significant differences were observed between the individuals of the 1996 year-class collected in the Gulf of Maine and the Gulf of St. Lawrence ($\chi^2 = 18.00$; $p = 0.049$).

Pairwise divergence

Significant differentiation was found between the southern and the European samples ($\phi_{ST} = 0.177$, $p = 0.05$; Table 6). Another significant difference was observed between the two samples of the southern populations comprising individuals from 1996 and 1997 in the Gulf of Maine and 1999 from North Carolina ($\phi_{ST} = 0.072$, $p = 0.02$).

DISCUSSION

The general genetic diversity characteristics of the Northwest Atlantic mackerel observed in the present study are comparable to those observed in two recent studies on the population structure of the Atlantic mackerel in the Northeast Atlantic and the Mediterranean Sea (Nesbo et al. 2000; Zardoya et al. 2004). The nucleotide diversity values, although slightly higher, coincide with those found for the Atlantic mackerel sampled in the Northeast Atlantic, the Mediterranean Sea and the 18 Canadian Atlantic mackerel included in these studies (Nesbo et al. 2000; Zardoya et al. 2004). In addition, the high values of Atlantic mackerel haplotype diversity observed in the present study are congruent with the values, ranging from 0.948 to 1.00, found by Nesbo et al. (2000) and Zardoya et al. (2004). Extreme haplotypic diversity of 1.00 was observed for the 18 Atlantic mackerel sampled in Canadian waters (Nesbo et al. 2000). In the present study, the estimated haplotype diversity of 0.984 is very high and indicates the presence of several unique haplotypes in the Northwest Atlantic. Such values are consistent with data obtained on globally distributed percid fish (e.g. Alvarado Bremer et al. 1995; Ward et al. 1995; Rosel and Bloc 1996) and are in agreement with theoretical mtDNA haplotype diversity expectations that are based on large population sizes (Hausser and Ward 1998).

Recent studies have shown that Atlantic mackerel from the eastern and western Atlantic have diverged despite an estimated coalescence time of only 5000 years (Scoles et al. 1998; Nesbo et al. 2000; Zardoya et al. 2004). Furthermore, genetic differentiation was detected among different regions of the Northeast Atlantic (Nesbo et al. 2000; Zardoya et al. 2004). In the present study, no such differentiation could be detected between the northern and southern populations of the Northwest Atlantic. The phylogenetic tree based on individuals from the 1999 year-class yielded a tree in which Atlantic mackerel from both northern and southern regions mixed with no apparent order. Likewise, the minimum spanning network based on all sampled haplotypes did not show any grouping of phylogenetically related haplotypes into the northern or southern regions (Fig. 4). These results are supported by those of the AMOVA and homogeneity tests carried out in the present study. The results of the present study are in agreement with those from previous studies showing no genetic divergence between the two populations of Atlantic mackerel (MacKay 1967; Maguire et al. 1987). Different hypotheses may be invoked to explain the lack of genetic differentiation between the northern and the southern populations of Atlantic mackerel. As mentioned above, the coalescence time of 5000 years has been estimated between the Northeastern and Northwestern Atlantic populations (Scoles et al. 1998). The lack of population differentiation may reflect the recent establishment of the southern and the northern populations of Atlantic mackerel in the Northwest Atlantic. A second more likely hypothesis is that mixing between the two populations is high enough to prevent genetic differentiation. Such mixing may take place during school formation and migration (MacKay 1967; Beckett et al. 1974; Parsons and Moores 1974; Moores et al. 1975; Stobo 1976). Such mixing between the two populations may not be a general occurrence but may be sufficient to prevent genetic divergence between the two populations. Waters et al. (2000*a*) estimated that straying of as little as 1% in large populations may result in a substantial gene flow between populations thus preventing genetic differentiation. Some mixing may also occur during migration when the northern individuals travel through the southern spawning areas when they leave the northern region to over-winter in warmer waters along the edge of the continental shelf from Sable Island to Long Island and when they migrate back to their spawning sites in spring (Sette 1950; Ware and Lambert 1985). Migration periods of the fish from both populations also overlap, leading to the possibility that early migrating southern individuals can meet late-migrating northern individuals and vice-versa (MacKay 1967). Sette (1950) also showed that Atlantic mackerel from the northern region intermingled with the southern fish in the Gulf of Maine area in summer via straying post-spawning individuals. In the present study, the GM98 sample most likely comprises fish from both regions since it was located in this mixing area and was sampled after the spawning season. Mixing between the two populations may also be favoured when an exceptionally strong recruitment take place. The significant genetic difference detected between year-classes prior to 1999 in both regions may be related to the smaller population size of these specific year-classes. This may explain the observed heterogeneity between fish of the northern and southern 1996 year-classes (χ^2 = 18.00, p=0.049). In contrast, the 1999 year-class is considered to be of exceptional size, thus having a high potential for expansion in the geographic distribution and hence of increasing the importance of gene flow between the northern and the southern populations. Therefore, regardless of the high inter-individual variability, a significant

amount of variation was detected between year-classes by both the AMOVA analyses and the homogeneity tests. A significant 4.24% of the variance in the Northwest Atlantic mackerel samples was due to differences between year-classes (Table 4). The homogeneity tests have shown that this variation results from a difference between haplotypes of fish belonging to different year-classes sampled in the Gulf of Maine and the Gulf of St. Lawrence.

Finally, our capacity to detect genetic structuring in the present study has been limited by the small number of individuals analyzed. Indeed, the variability of the left branch of the D-loop is very high in Atlantic mackerel (Nesbo et al. 2000; Zardoya et al. 2004; this study) and a larger number of individuals should have been analyzed in order to take advantage of the high discriminating power of this genetic marker. Additional sampling concentrating on spawning individuals should be carried out in future studies.

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Table 1 Atlantic mackerel (Scomber scombrus L.). Description of the samples selected for the population genetic study based on the sequence analyses of the mtDNA control region. N = sample size; s.d. = standard deviation.

Population	Sampling site (Abbreviation)	Sampling date	Longitude	Latitude	Year-class	*N*	Mean size in mm (s.d.)
Northern	Gulf of St. Lawrence (GL98)	06/07/1998	64°15'47	48°27'60		21	314.7 (27.2)
					1996	10	
					1995	8	
					1994	3	
	Gulf of St. Lawrence (GL01)	22/06/2001	64°45'00	48°12'00	1999	16	278.8 (47.8)
Southern	Gulf of Maine (GM98)	24/07/1998	68°25'05	44°17'19		18	257.3 (20.7)
					1997	9	
					1996	9	
	North Carolina (NC01)	4/02/2001	75°22'18	36°49'66	1999	20	231.8 (9.7)

Note: For the GM98 sample, the mtDNA sequence was determined on 19 specimens. Since the age of one individual of haplotype 4 was not determined, *N* = 18 in this table.

Table 2. Atlantic mackerel (*Scomber scombrus* L.). Frequencies of the mtDNA haplotypes for each sample and year-class collected in the Northwest Atlantic. Sample site abbreviations are given in Table 1. *N* = sample size.

Haplotype	Southern population			Northern population			
	GM98		NC01	GL98			GL01
	1996	1997	1999	1994	1995	1996	1999
1	0	0	1	0	2	0	0
2	2	0	0	1	0	0	1
3	0	0	1	0	0	1	1
4	1	0	1	0	0	1	1
5	0	1	0	0	1	0	0
6	0	0	0	0	0	1	1
7	0	1	2	0	0	0	0
8	1	0	1	0	2	0	0
9	0	0	0	0	0	1	2
10	0	0	0	0	0	2	0
11	2	0	0	0	0	0	1
12	0	0	2	0	0	0	0
13	0	0	1	0	0	0	1
14	0	0	0	0	0	1	1
15	0	1	1	0	0	1	0
16	0	0	1	0	0	0	0
17	0	0	1	0	0	0	0
18	0	0	1	0	0	0	0
19	0	0	1	0	0	0	0
20	0	0	1	0	0	0	0
21	0	0	1	0	0	0	0
22	0	0	1	0	0	0	0
23	0	0	1	0	0	0	0
24	0	0	1	0	0	0	0
25	0	0	1	0	0	0	0
26	0	1	0	0	0	0	0
27	1	0	0	0	0	0	0
28	1	0	0	0	0	0	0
29	1	0	0	0	0	0	0
30	0	1	0	0	0	0	0
31	0	1	0	0	0	0	0
32	0	1	0	0	0	0	0

Table 2. (Continued).

Haplotype	Southern population			Northern population			
	GM98		NC01	GL98			GL01
	1996	1997	1999	1994	1995	1996	1999
33	0	1	0	0	0	0	0
34	0	1	0	0	0	0	0
35	0	0	0	0	0	1	0
36	0	0	0	0	0	1	0
37	0	0	0	0	1	0	0
38	0	0	0	0	1	0	0
39	0	0	0	1	0	0	0
40	0	0	0	1	0	0	0
41	0	0	0	0	1	0	0
43	0	0	0	0	0	0	1
44	0	0	0	0	0	0	1
45	0	0	0	0	0	0	1
46	0	0	0	0	0	0	1
47	0	0	0	0	0	0	1
48	0	0	0	0	0	0	1
49	0	0	0	0	0	0	1
N	9	9	20	3	8	10	16

Note: For the GM98 sample, the mtDNA sequence was determined on 19 specimens. Since the age of one individual of haplotype 4 could not be determined, $N = 18$ in this table.

Table 3. Atlantic mackerel (*Scomber scombrus* L.). Haplotype (H) and nucleotide (π_n) diversities of the samples collected in the Northwest Atlantic. Two sequences of the Northeast Atlantic mackerel are also included. Sample site abbreviations are given in Table 1. N = sample size.

Sample and Region	*N*	Haplotype diversity (H)	Nucleotide diversity (π_n)
GL98	21	0.986	0.027
GL01	16	0.992	0.026
Northern region	37	0.983	0.026
GM98	19	0.982	0.028
NC01	20	0.989	0.029
Southern region	39	0.985	0.029
Northwest Atlantic	76	0.984	0.030
Northeast Atlantic	2	—	—000
Overall	78	0.984	0.028

Table 4. Atlantic mackerel (*Scomber scombrus* L.). Analysis of molecular variance on individuals nested by year-class and region: the first analysis considers all samples while the second considers all samples except GM98. Sample site abbreviations are provided in Table 1. d.f. = degrees of freedom.

	Observed partition				
Variance component	d.f.	Variance	% total	P^a	Φ-statistic
All samples					
Between northern and southern regions	1	-0.06865	-1.61	0.778	Φ_{CT} = -0.016
Between year-classes within each region	5	0.18108	4.24	0.037	Φ_{SC} = 0.042
Between individuals within each year-class	68	4.15438	97.37	0.053	Φ_{ST} = 0.026
All samples except GM98					
Between northern and southern regions	1	0.03628	0.84	0.400	Φ_{CT} = 0.008
Between year-classes within each region	3	0.12741	2.94	0.109	Φ_{SC} = 0.030
Between individuals within each year-class	52	4.16325	96.22	0.074	Φ_{ST} = 0.038

P^a: Probability of finding a more extreme variance component and ϕ-statistic than the observed values by chance alone.

Table 5. Atlantic mackerel (*Scomber scombrus* L.). Results of the homogeneity tests carried out between specific year-classes for estimation of temporal and geographical variabilities. $\chi 2$ = chi-square value calculated from the original matrix; p = probability of exceeding original $\chi 2$ value by chance.

Samples compared	χ^2	p
Year-classes 1995, 1996, 1999 from the Gulf of St. Lawrence (northern population)	53.27	0.176
Year-classes 1995 and 1996 from the Gulf of St. Lawrence (northern population)	18.00	0.001
Year-classes 1996, 1997, 1999 from the Gulf of Maine and North Carolina (southern population)	62.54	0.256
Year-classes 1996 and 1997 from the Gulf of Maine (GM98)	18.00	$p<0.001$
Year-class 1999 from the Gulf of St. Lawrence (northern population) and North Carolina (southern population)	29.93	0.370
Year-class 1996 from the Gulf of St. Lawrence (northern) and the Gulf of Maine (southern population)	18.00	0.049

Table 6. Atlantic mackerel (*Scomber scombrus* L.). Pairwise divergence statistics (ΦST (p)) calculated for the southern and northern populations as well as for the different samples from the different populations. Individuals from Northeast Atlantic (European population) are also included.

		I	II	III	IV	V	VI
I	Southern population						
II	NC01	—					
III	GM98	—	0.072 (0.02)				
IV	Northern population	0.002 (0.35)	0.027 (0.07)	0.019 (0.13)			
V	GL98	-0.004 (0.51)	0.022 (0.13)	0.012 (0.25)	—		
VI	GL01	-0.001 (0.42)	0.022 (0.17)	0.019 (0.22)	—	-0.014 (0.71)	
VII	European population	0.177 (0.05)	0.190 (0.05)	0.232 (0.04)	0.184 (0.07)	0.184 (0.09)	0.195 (0.07)

Figure 1. Atlantic mackerel (*Scomber scombrus* L.). Geographic locations of the sampling sites in the Gulf of St. Lawrence, in the Gulf of Maine and off the coast of North Carolina. Sample site abbreviations are given in Table 1.

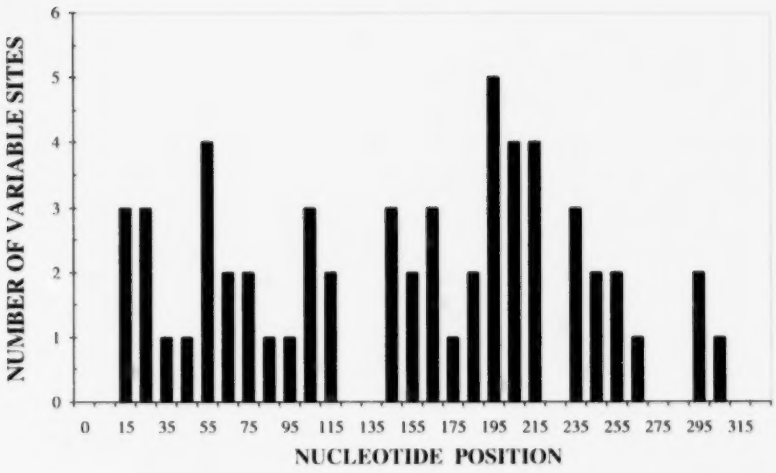


Figure 2. Atlantic mackerel (*Scomber scombrus* L.). Location and extent of the variability detected in the mtDNA control region as measured by the number of variable sites per base pair fragment among 76 sequences.

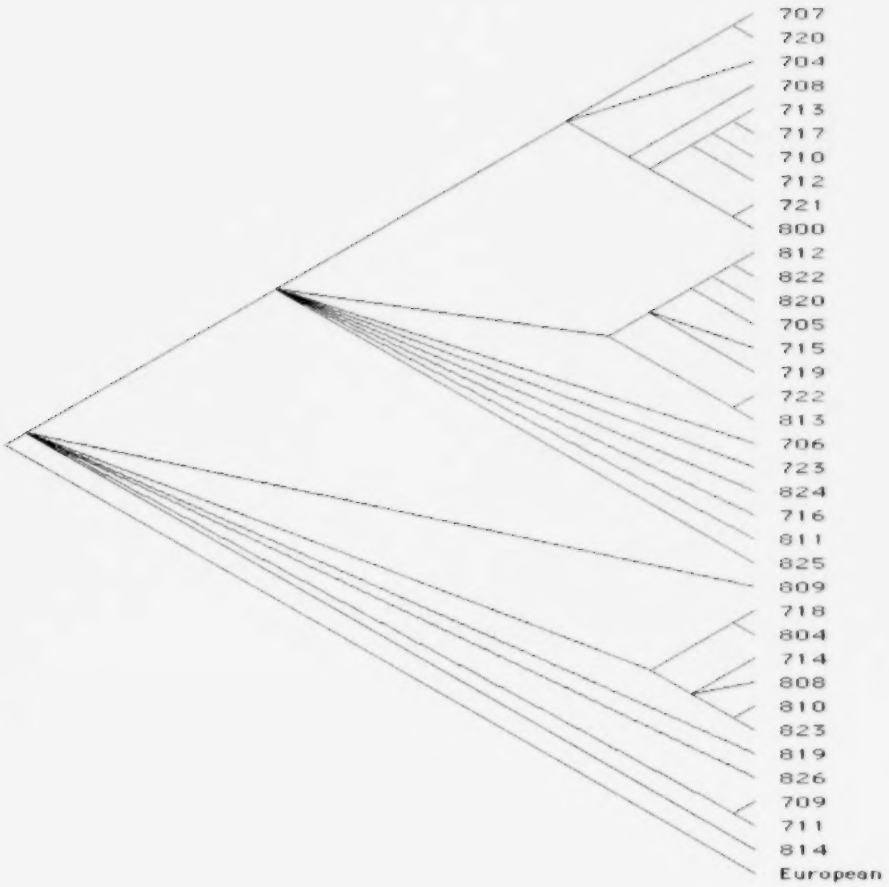


Figure 3. Atlantic mackerel (*Scomber scombrus* L.). Maximum likelihood phylogenetic tree of individuals of the 1999 year-class collected in the Gulf of St. Lawrence (GL01) and off the North Carolina coast (NC01) in 2001. Fish numbers beginning with 7 identify fish from the North Carolina coast while those beginning with 8 correspond to fish from the Gulf of St. Lawrence. A specimen from the Northeast Atlantic (European) is also included.

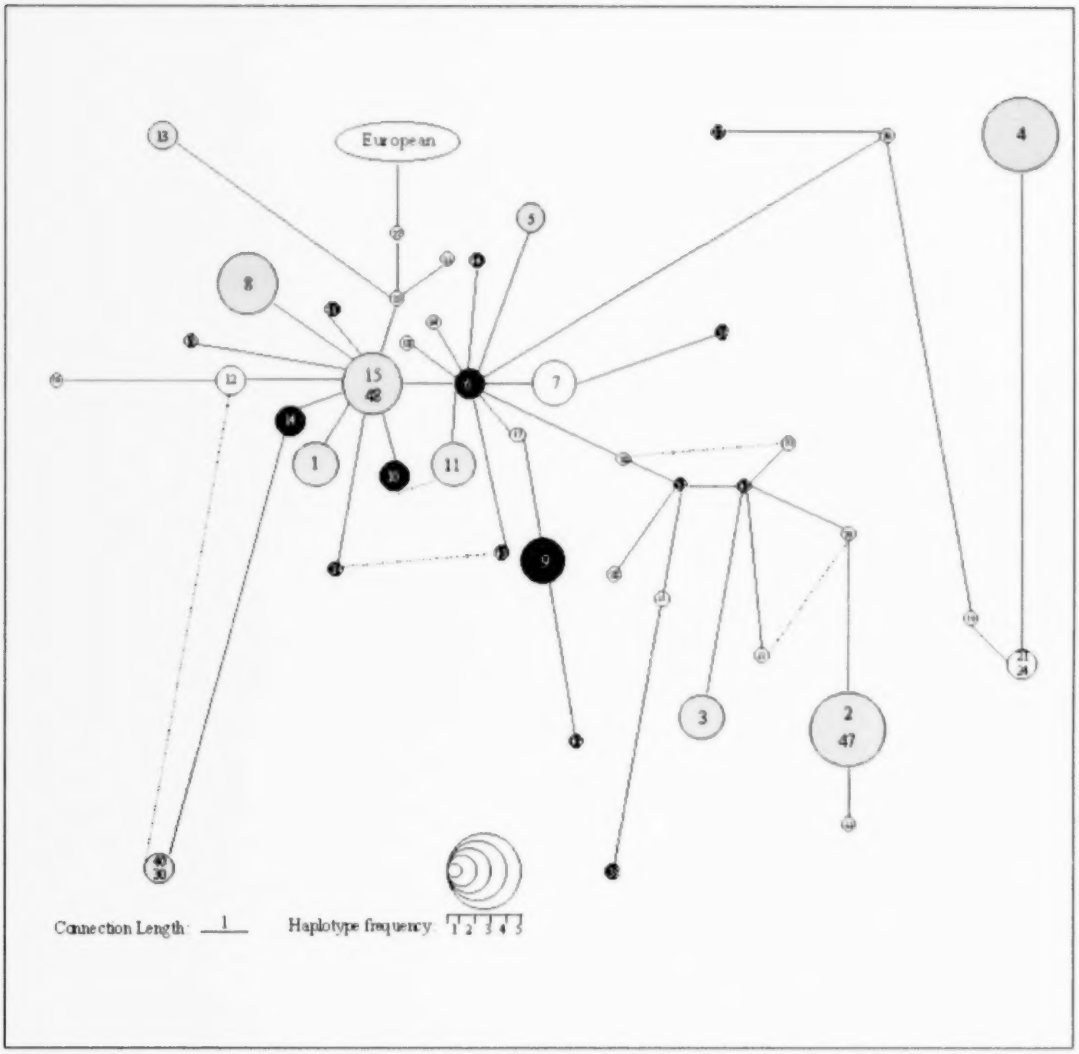
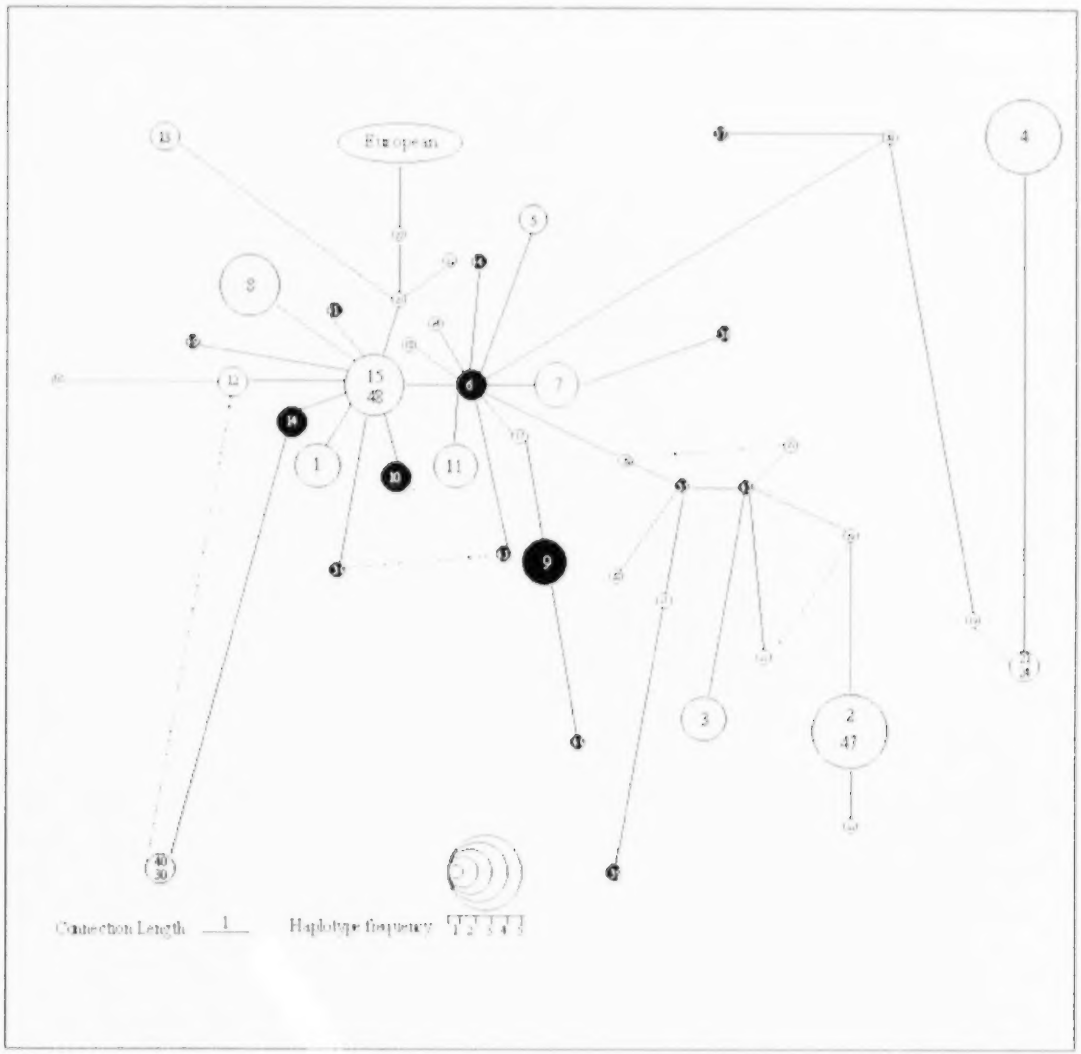


Figure 4. Atlantic mackerel (*Scomber scombrus* L.). Minimum spanning network describing the relationships between haplotypes observed in the Northwest Atlantic. The relationship with the haplotypes of Atlantic mackerel from the Northeast Atlantic (European) is also illustrated. Black circles correspond to haplotypes found in the northern population, white circles to those found in the southern population and grey circles to those found in both populations. The size of the circle is proportional to number of individuals sharing a particular haplotype. The number inside the circle corresponds to the identification number of the haplotype. In some cases, the calculation parameters yielded null distances between haplotypes, thus pooling them on the network.



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